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14. ABSTRACT
The acquisition of the mass spectrometer has been a game changer when it comes to product isolation and identification. This instrument has been an asset in organic synthesis and natural product isolation and teaching in organic, biochemistry, and instrumental analysis classes. Over the last year thie mass spectrometer has directly influenced the research projects of approximately 18 undergraduate and graduate students and the course work of an additional 32 undergraduate students. This summer this instrument will affect the research projects of an additional 15 students will be directly affected by this instrument. This instrument has been used in organic

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Report Title

Final Report: Acquisition of HPLC-Mass Spectrometer

ABSTRACT

The acquisition of the mass spectrometer has been a game changer when it comes to product isolation and identification. This instrument has been an asset in organic synthesis and natural product isolation and teaching in organic, biochemistry, and instrumental analysis classes. Over the last year thie mass spectrometer has directly influenced the research projects of approximately 18 undergraduate and graduate students and the course work of an additional 32 undergraduate students. This summer this instrument will affect the research projects of an additional 15 students will be directly affected by this instrument. This instrument has been used in organic research, natural product identification, and teaching.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

"Microwave synthesized succinimides purified by flash chromatography" Guetzloff, Thomas F.; Dudding, Bridgett; Fultz, Micheal. 249th National ACS meeting, Denver, CO. CHED-670.

"Synthesizing and trialing triesterified monosaccharides for protected culture pest control" Cavender, Hannah; Fultz, Michael W.; Guetzloff, Megan; Liedl, Barbara. 249th National ACS meeting, Denver, CO. ORGN-153.

"Microwave synthesis of N-phenyl succinimides and malenamides in undergruate organic chemistry laboratory" Guetzloff, Thomas F.; Dudding, Bridgett; Guetzloff, Megan; Fultz, Michael W. 249th National ACS meeting, Denver, CO. CHED-1321.

"Synthesis of capsaicin analogs" Slater, Tabatha; Higginbotham, Ethan; Fultz, Michael W. 249th National ACS meeting, Denver, CO. CHED-1103.

"Progress toward the synthesis of (S)-curvularin" Fultz, Michael W.; Slater, Tabatha. 249th National ACS meeting, Denver, CO. CHED-1102.

Number of Presentations: 5.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

2015 Passer Award from the American Chemical Society to attend a conference at the end of May to optimize the activities on the mass spectrometer.

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

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Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

The installation of this instrument was finally completed in January so there has not been a lot of time to have significant breakthroughs. We have been working on projects relating to insecticides for green house vegetable production, tomato volatile production, capsaicin analog synthesis for studies on non-small cell lung cancer, microwave synthesis of succinimides, and the synthesis of gliotoxins and sumarlarins. This instrument has been used for intermediate structure confirmation and identification.

Technology Transfer

West Virginia State University
Mass Spectrometer Progress report

Each of these research projects used the mass spectrometer to confirm structures and characterize intermediates. Details of the project and plans to complete those projects are included in the description.

Screening Vintage Tomato Varieties with CAPS, SCAR and SNP Markers for Disease Resistance

Investigator: Barbara E. Liedl, West Virginia State University, Gus R. Douglass Institute, Agricultural and Environmental Research Station

Tomato production in protected culture has been one of the fastest growing industries with a 600% increase in a fifteen-year period. West Virginia State University has a tomato breeding program focused on developing lines with insect and disease resistance as well as improved organoleptic traits for this production system. Consumers are interested in buying vintage tomatoes, but these varieties do not have resistance to diseases as found in most modern varieties. We could transfer these traits; however, before we can transfer these traits using marker assisted selection, we need to evaluate the vintage lines with their markers to verify we can use the molecular markers to transfer the traits. To accomplish this, twelve vintage tomato varieties along with disease resistant germplasm acquired from the Tomato Genetics Resource Center will be screened for CAPS, SCAR and SNP markers for two diseases – Fusarium and Verticillium wilt. The goal of this project is to add to new markers to the existing database of molecular markers to use in breeding tomatoes for protected culture.

Marker details will be obtained from published reports included primers sequence, restriction enzymes and the expected restriction products[i]. DNA will be isolated and checked for quality from fresh and/or frozen plant tissue of vintage cultivars as well as lines possessing the trait of interest as a control. Each marker will be amplified from DNA using PCR. If needed the PCR amplicon will be digested with the appropriate restriction enzyme prior to electrophoresis. Scoring will be done using the size of the amplicons and restriction products on agarose electrophoretic gels with a molecular size standard for comparison. Modifications in PCR conditions may be necessary to obtain optimal PCR amplification and in some cases, several restriction enzymes may need to be evaluated. The data obtained from these markers will be entered into an existing database that will be used for marker assisted selection in the development of tomato cultivars with improved traits.

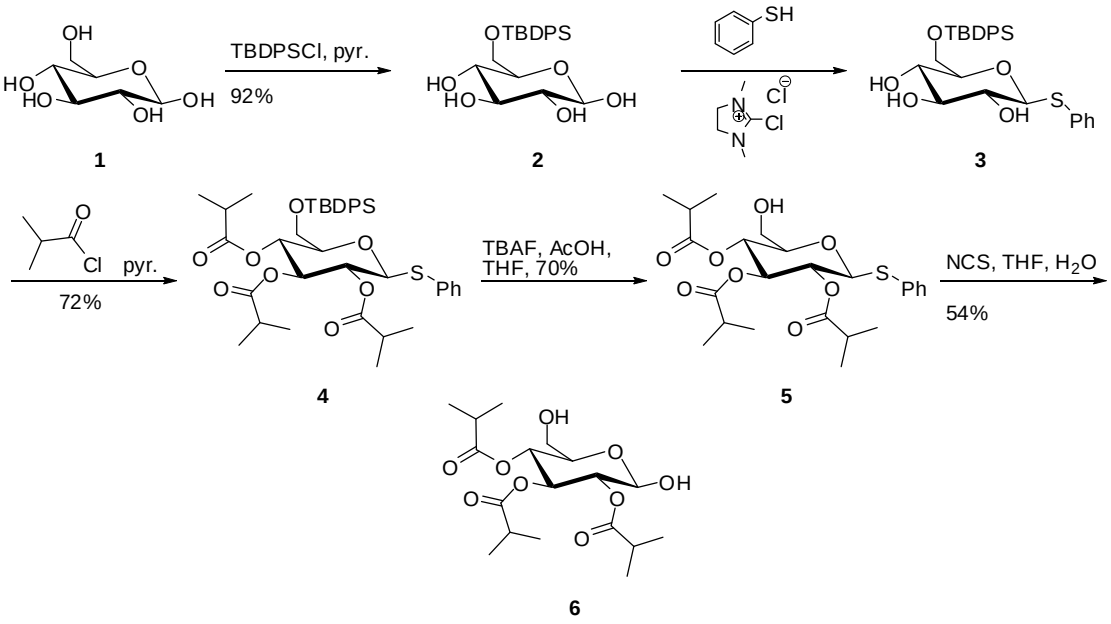
Synthesis of (2R,3R,4S,5R,6R)-2-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methylpropanoate)

Investigator: Micheal Fultz, West Virginia State University, College of Natural Science and Mathematics, Department of Chemistry

Even with the use of integrated pest management (IPM), control of insects in agriculture relies heavily on pesticides, a practice that is increasingly limited by evolution of pesticide-resistant insects and increased health and environmental concerns. Several pests including, whiteflies (*Bemisia tabaci* (Gennadius) or *Bemisia argentifolii* Bellows & Perring*;* sweetpotato whitefly B biotype or silverleaf whitefly) are difficult to eliminate with chemical or biological methods.[ii] However, acylsugars exuded from type IV glandular trichomes of *S. pennellii* mediate the resistance of *S. pennellii* to several pest species.[iii] The non-toxicity and broad spectrum of the

resistance are major advantages for use in production. Field tomatoes with acyl-sugar mediated insect resistance are being developed at Cornell University. However, one major consideration in using this resistance in protected culture (greenhouse and high tunnel) is the effect of acylsugars on beneficial insects used in greenhouse production.

The synthesis of these potential insecticides begins with a hexose **1** (**Scheme 1**). Protection of the anomeric position[iv] will provide the stabilized sugar **2**. Kinetic protection of the primary alcohol reveals the triol **3** that are to be esterified to provide the fully protected sugar. This sugar would then be treated to deprotect and provide **5. D**eprotection of the anomeric position reveals the desired monosaccaride derivative **6.** The acid chlorides can be exchanged with propionyl chloride, octanoyl chloride, decanoyl chloride, and dodecanoyl chloride to provide all of the desired monosaccaride triesters needed for agricultural studies.



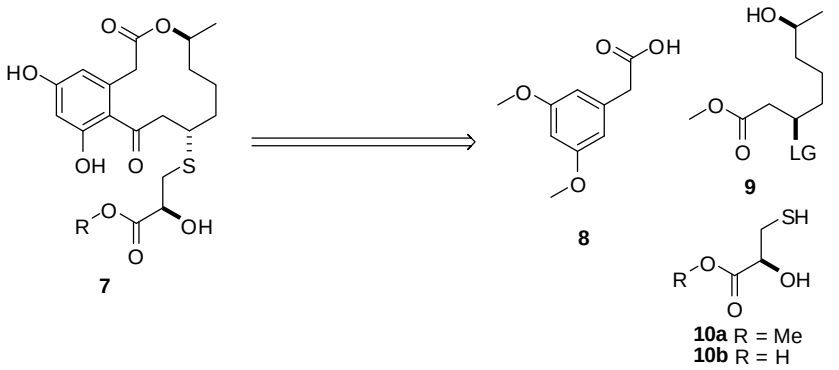
Scheme 1 Esterification of monosaccarides

This project has been completed with full characterization of each of the intermediates have been isolated, characterized, and stored. The final products have moved on to Dr. Liedl for examination of the acyl sugar as an alternative to traditional insecticides in greenhouses.

Progress towards the synthesis of sumarlarin A

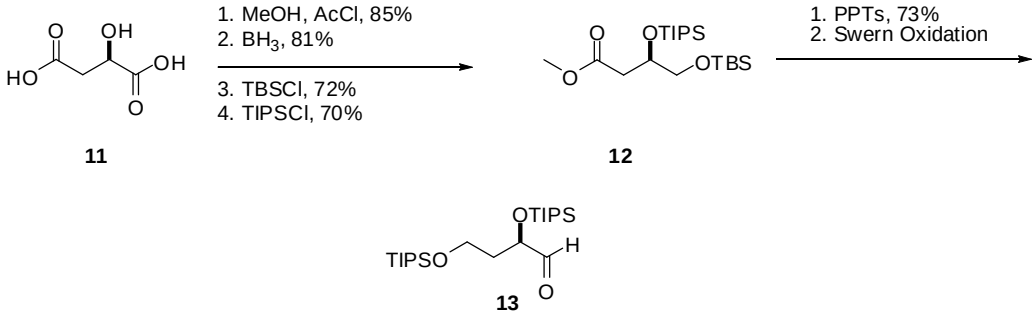
Investigator: Micheal Fultz, West Virginia State University, College of Natural Science and Mathematics, Department of Chemistry

The progress towards the synthesis of sumarlarin A (**7**) is displayed in the reaction schemes below. Total synthesis of any natural product is a low process requiring careful study of the reaction, modifying the reaction to improve yields and verifying each product structure. The retrosynthesis of sumarlarin A to its component fragments can be seen through a hydrolysis of the lactone and cleavage of the aromatic ketone to provide advanced intermediates **8** – **10**.



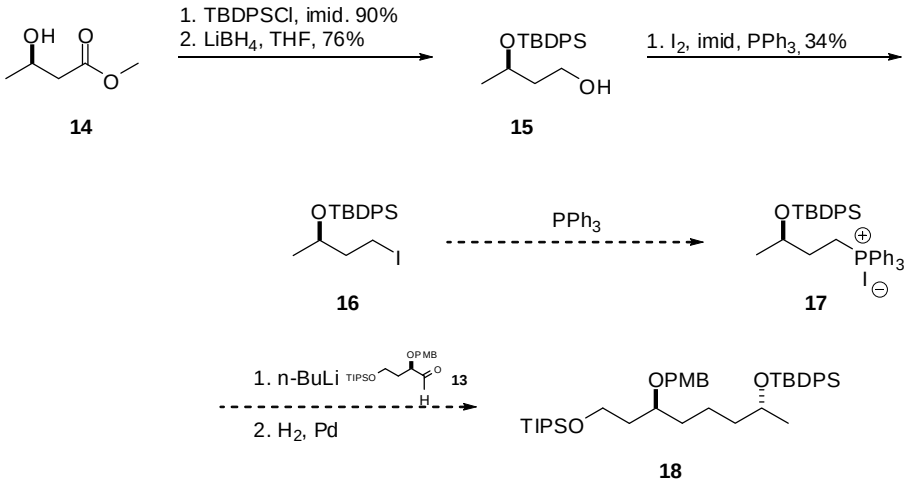
Scheme 2 Retrosynthesis of Sumarlarin A

Structure number **8** is commercially available so no synthetic efforts are needed. Work has focused on developing a coherent route to structure **8**. This work focuses in on the construction of aldehyde **13** which has been completed as shown in **Scheme 3** and the phosphonium salt **12** (**Scheme 4**). Aldehyde 7 has been formed from the oxidation of primary alcohol but only in a test batch to prevent the epimerization of the chiral center.



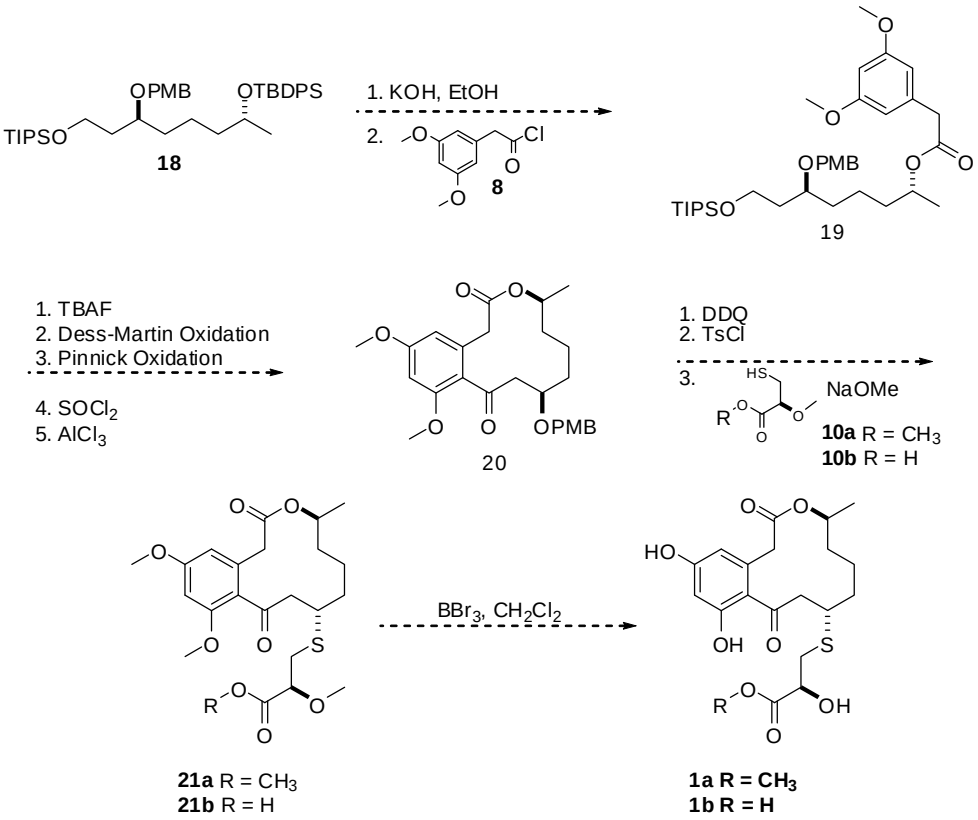
Scheme 3 The synthesis of aldehyde **13**

The phosphonium salt has been a little more difficult to construct where current attempts to form the primary iodide from the alcohol has been difficult. We have recovered low yields of the iodide, with unreacted starting material, and the elimination product as a mixture.



Scheme 4 Efforts to complete Fragment **9**.

Plans to complete the natural products are shown below.



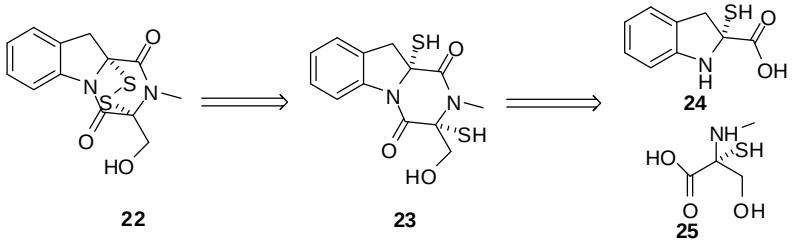
Scheme 5 Plans to complete sumarlarin A

Progress towards the synthesis of 6-deoxy-5a,6-didehydrogliotoxin

Investigator: Micheal Fultz, West Virginia State University, College of Natural Science and Mathematics, Department of Chemistry

6-deoxy-5a,6-didehydrogliotoxin was isolated from the other six metabolites in *Penicillium* sp. (strain JMF304), 6-Deoxy-5a,6-didehydrogliotoxin (**22**) was found to have the molecular formula $C_{13}H_{12}N_2O_3S_2$ from HR ESIMS and NMR data. The H NMR data suggests the presence of four aromatic protons, a pair of nonequivalent methylene protons, an N-methyl, and a hydroxymethyl group. The COSY data suggested that the benzene ring was *ortho* disubstituted. The remaining signals matched up with those of dehydrogliotoxin.

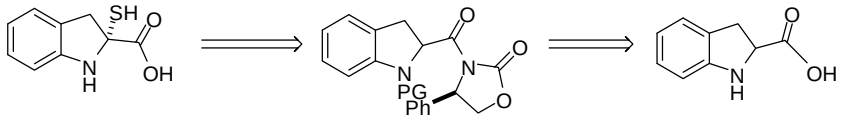
The target product 6-deoxy-5a,6-didehydrogliotoxin (**22**) can be dissected at the disulfide bridge to provide the intermediate **23** (**Scheme 1**). This intermediate can further be broken down by the hydrolysis of the amide bonds to provide the two target intermediates **24** and **25** needed for the coupling experiments to complete the natural product followed by cleaving the amide bonds which gives the intermediates **24** and **25**. **24** was the target fragment for this research.



Scheme 6 Retrosynthetic of **22**

Synthetic efforts for fragment 24

Fragment **10** can be formed from the acylation of indoline-2-carboxylic acid **13** to give the Evans chiral auxiliary **12**.



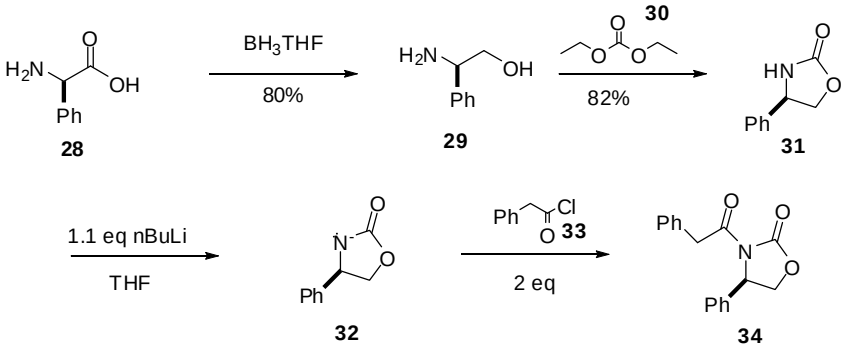
24 **26** **27**

Scheme 7 Retrosynthetic analysis of fragment **24**

Examining the Chiral Sulfonation

Attempt 1 for Examination of Evans Chiral Auxiliary

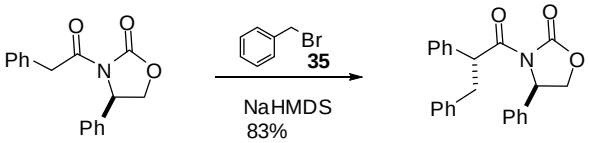
To begin the studies of the chiral sulfonation standard conditions had to be developed (**Scheme 8**). This work began with the synthesis of the Evan Chiral Auxiliary. The work began with phenyl alanine full reduction of the acid provided the aminol, cyclization to provide the auxillary occurred smoothly using to provide the auxiliary. Acylation occurred upon deprotonation of the nitrogen and quenching with the acid chloride to provide the initial test substrate needed for electrophilic substitutions.



28 **29** **31** **32** **34**

Scheme 8 Examination of Evans Chiral Auxiliary

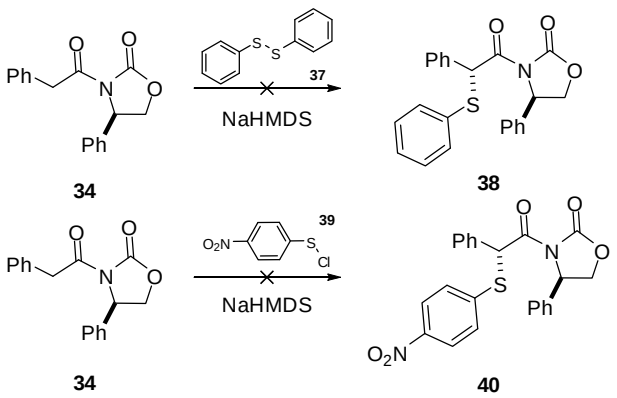
The reactivity of this substrate began with the deprotonation of the alpha proton to form the chiral enolate **34** (**Scheme 9**). This was quenched with 4-nitrobenzyl bromide to provide the chiral alkylated product in good yield (83%) and moderate diastereoselectivity (5:1 ratio)



34 **36**

Scheme 9 Alkylation of the Evans Chiral Auxiliary

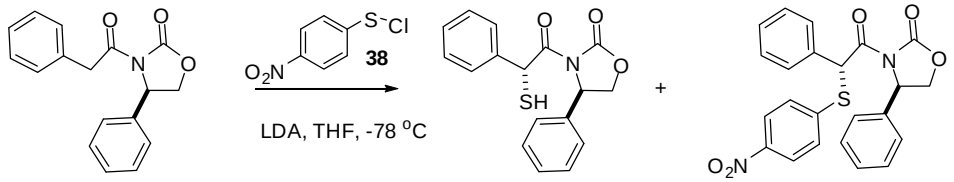
With the chiral alkylation reaction shown to work and being a known reaction attention shifted to the unknown (**Scheme 10**). Two separate electrophilic sources of sulfur were used in the attempted chiral sulfonation. *P*-nitrophenyl sulfenyl chloride and phenyl disulfide were used in this reaction. However neither reaction was successful in providing the sulfonated product in any appreciable amount. With the successful installation of the Mass spectrometer a new study of this reaction will begin to determine if any of the desired product was formed .



34 **38** **34** **40**

Scheme 10 Attempt at Synthesizing Fragment 10

The sulfonation (**Scheme 11**) carried out with the electrophilic sulfur source, 4-nitrophenylsulfenyl chloride (**38**). While the sulfur acts as an electrophile the aromatic ring can undergo nucleophilic aromatic substitution. This could also lead to potential by-products like **41** minimizing the yield of the desired sulfide, increasing the polarity of the product and making it difficult to isolate. The use of the newly acquired liquid chromatograph-mass spectrometer will allow for the full characterization of reaction solutions to determine if any of these side reactions actually occurred.

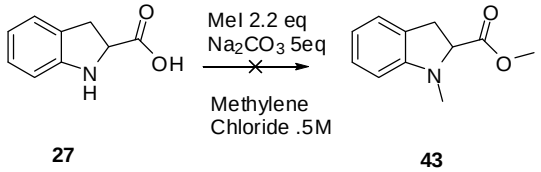


34 **41** **42**

Scheme 11 Sulfonation of Evans Auxiliary

Due to the fact that neither of these sulfonations appear to work under any of the conditions that we attempted we turned our attention to more standard alkylation procedures. To begin this study the nitrogen needed to be protected. The standard amine protecting group *tert*-butoxy carbamate is too sterically hindered to act as a nucleophile.

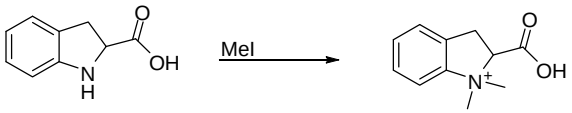
The first attempted at forming the tertiary sulfide in an achiral pathway is by the methylation of fragment **27** was carried out under the conditions in **Scheme 12**. Ideally the methylation of Indoline-2-carboxylic acid would provide a product that would be capable of producing an enolate for the synthesis of the chiral auxiliary, but this reaction failed.



27 **43**

Scheme 12 First Attempt at Synthesis of Fragment **24**

Indoline-2-carboxylic acid was treated with iodomethane and sodium carbonate (5 eq) in the presence of methylene chloride. The product was never able to be isolated. This was most likely due to the over methylation of the nitrogen to provide the ions that are water soluble and removed in the aqueous workup (**Scheme 13**).



27 **44**

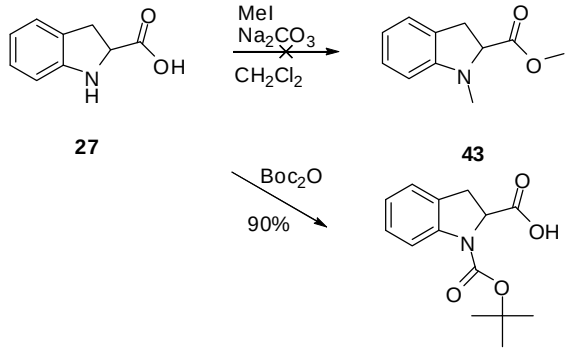
Scheme 13 Over Methylation of the Nitrogen

This over methylation of the nitrogen would lead to a positive charge on the nitrogen (**44**). This could react with water and the silica gel of the column and result in the product not eluting out of the column, prohibiting the product from being isolated.

Final Attempt of the Synthesis of Fragment 10

The final attempt of the synthesis of fragment **24** was the protection of the indole to give an acid that is capable of providing either the acid chloride needed to provide the Evans chiral auxiliary for chiral sulfonations or the ester needed for direct achiral sulfonations. (**Scheme 14**)

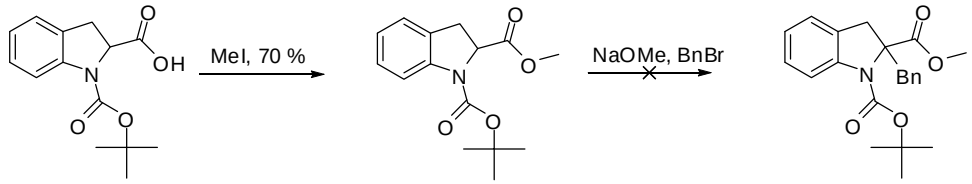
Indoline-2-carboxylic acid was treated with a Boc anhydride to provide **45** (**Scheme 14**). The product formed at a great 90% yield. The methyl ester was then synthesized using the previously purified methyl iodide to provide the ester needed for nucleophilic attack.



27 **43** **45**

Scheme 14 Final Attempt at Fragment 9: Protection of Indole

This sulfonation reaction did not proceed as planned. All attempts to sulfonate of alkylate the ester failed to provide any appreciable amount of product (**Scheme 15**). This failure maybe attributed to the large bulky *tert*-butyl carbamate blocking the approach of any electrophile. Replacing this large bulky *tert*-butyl carbamate with a thinner less sterically encumbering protecting group such as a methyl carbamate may eliminate the inhibition of the blocking protecting group and allow the alkylation to proceed and will be attempted when the protecting group arrives.

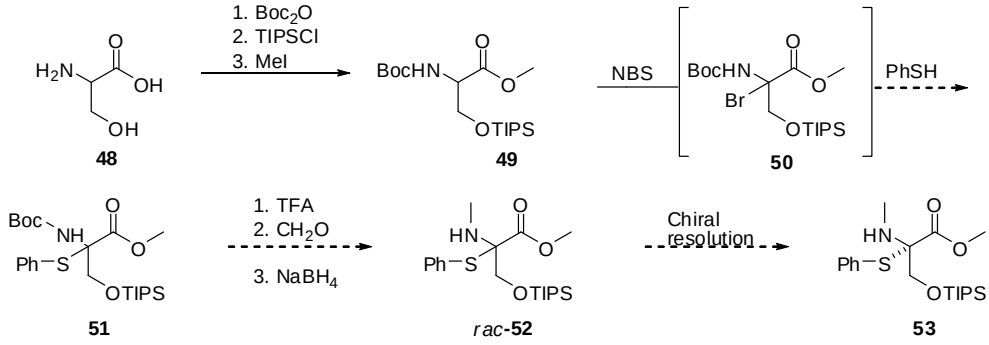


45 **46** **47**

Scheme 15 Alkylation attempts of diprotected indole 31

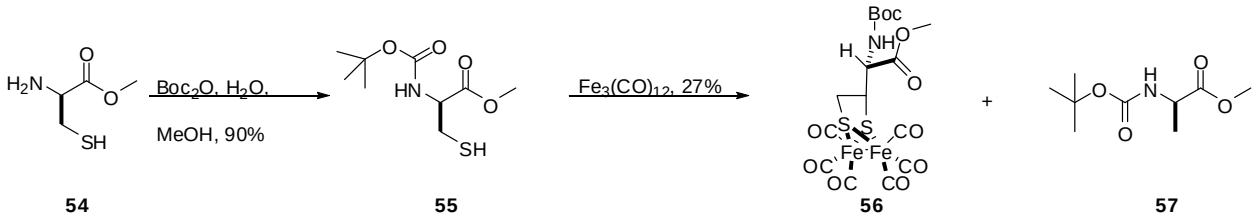
The second half of gliotoxin began with commercially available racemic N-methylserine (**Scheme 16**). The stereoselective incorporation of sulfur mentioned above will not work here

due to the elimination of the siloxide which would provide the undesired alkene. Protection of the functional groups followed by halogenation of the alpha proton provided the necessary starting material for the sulfonation reaction. Free radical bromination has been successful at incorporating bromine on carbons that include nitrogen.[v] Crude NMR of the brominated product showed the disappearance of the alpha proton, however attempts to displace the bromide yielded only the starting product.



Scheme 16 Formation of the serine derivative **11**

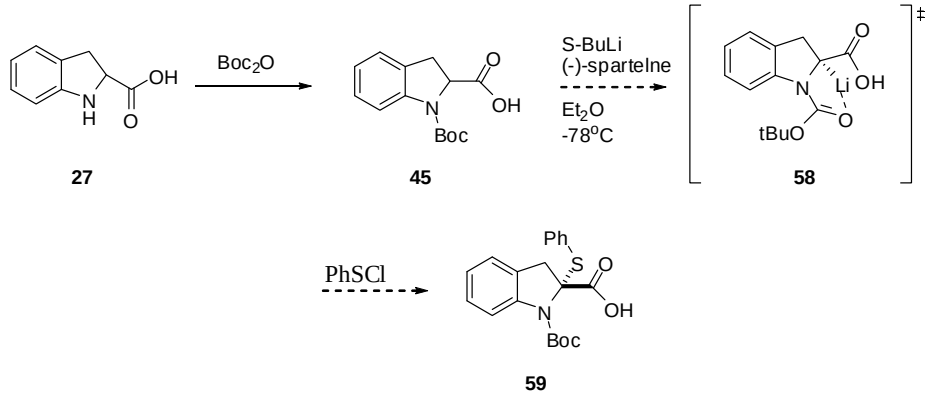
Work to incorporate the sulfur stereoselectively continued with the use of diiron dodecyl carbonyl to rearrange *l*-cysteine to provide the dithiol and phenyl alanine. This dithiol is coordinated to the iron and all attempts to decompose the ionic coordination complex **56** to recover strictly the dithiol failed.



Scheme 17 Stereoselective formation of dithiol **17**

Future work on the substitution at the alpha position of 13

Reaction **Scheme 13** provides a third possible sulfonation process of an asymmetric deprotonation providing a lithium complex with sparteine. This reaction scheme will also direct stereochemistry of the product to provide the substituted sulfide **46**.[vi] This is an additional pathway to study in future semesters.



Scheme 18 Further Sulfonation work

With further work, we hope that a route to a tertiary chiral sulfur will be found. Once the synthesis of the tertiary chiral sulfur is complete, the final product can be formed and used for further biological studies.

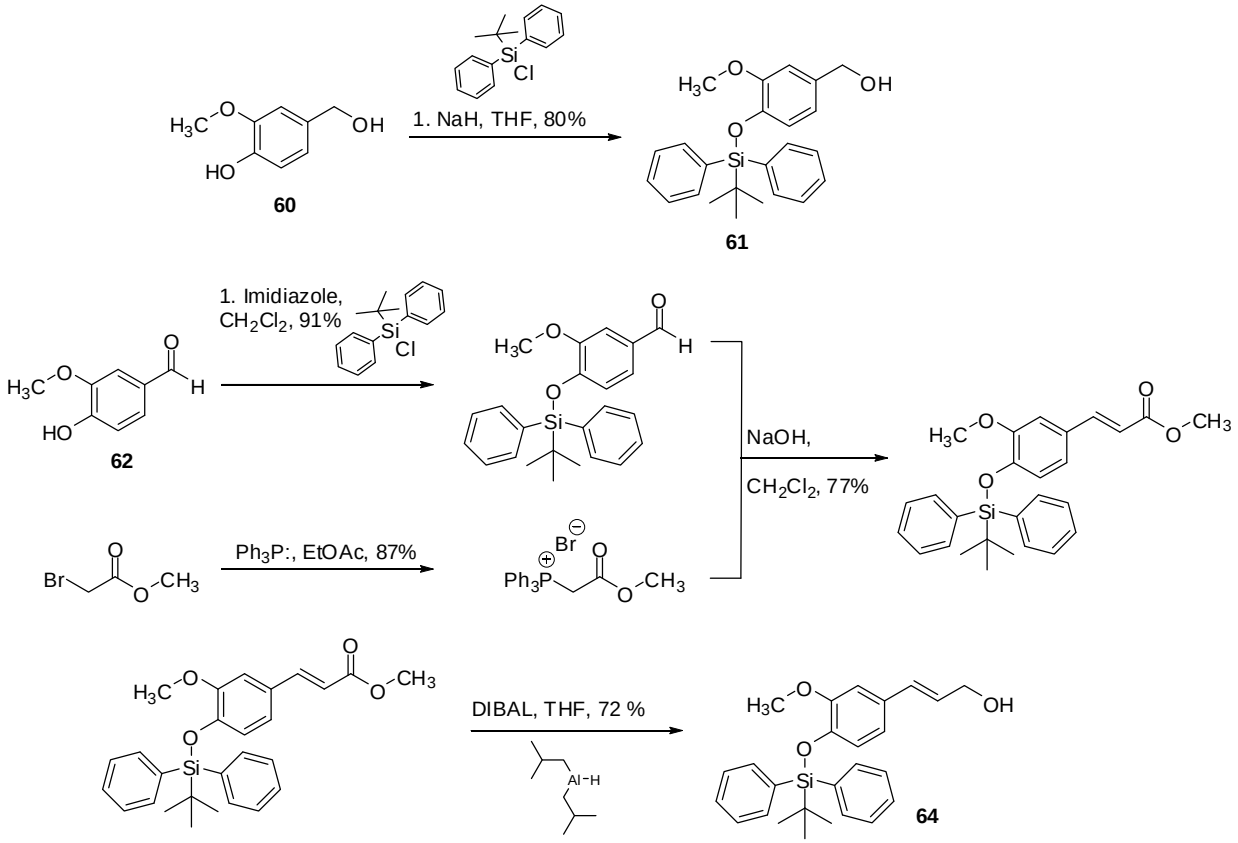
Synthesis of capsaicin analogs

Investigator: Micheal Fultz, West Virginia State University, College of Natural Science and Mathematics, Department of Chemistry

Capsaicin is the chemical found in peppers that causes a burning sensation when eaten and it is currently used in ointments for treatment of pain and inflammation.

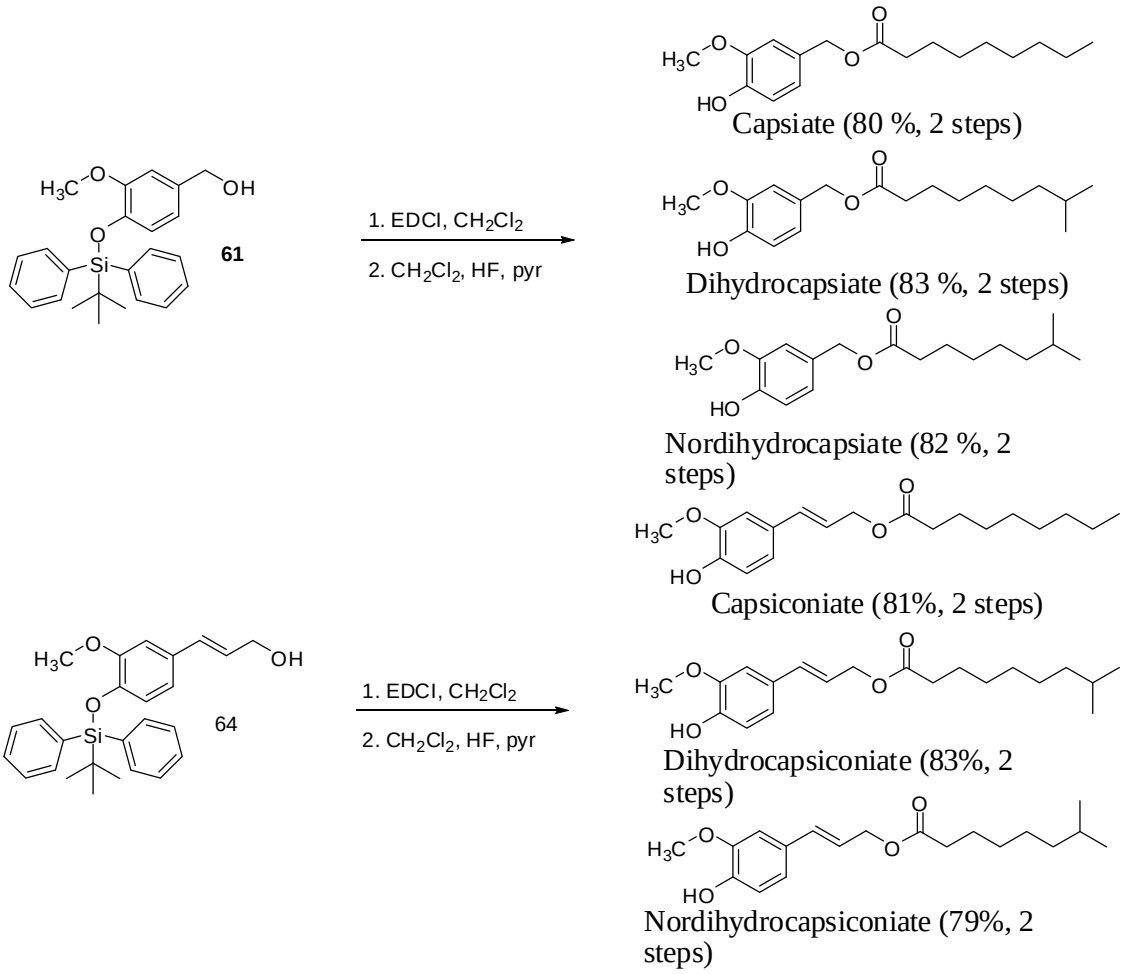
Capsaicin shows promise as an effective anti-cancer nutritional agent and is highly selective for several human cancer cells including non-small cell lung cancer, T-cell leukemia, prostate cancer, and colon cancer by causing apoptosis and cell cycle arrest in those cells but leaving healthy cells untouched.

To synthesize each analog, an allylic or benzylic alcohol was be coupled to commercially available saturated carboxylic acid. The alcohols were synthesized in a short, direct pathway from the commercially available phenol **60** or benzaldehyde **62**.



Scheme 19 Formation of the allylic and benzylic alcohols.

Coupling of the alcohol with the commercially available acids provides the carbon backbone of the target compounds. Pyrdinium fluoride removal of the silyl ether completes the desired analogs.



Scheme 20 Completion of the capsaicin analogs.

This project has been completed and the products have been sent to the Marshall University School of Medicine for biological activity.

All of these projects were significantly enhanced with the acquisition of the mass spectrometer.

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